THE ANAEROBIC GASEOUS METABOLISM OF THE ROACH, CRYPTOCERCUS PUNCTULATUS SCUDDER

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In the experiments of Davis and Slater (1926, 1928) on the anaerobic metabolism of the cockroach, *Periplaneta orientalis,* the oxygen debt incurred during anaerobiosis was paid off exactly, and the excess respiration of recovery appeared to be due to the complete oxidation of the lactic acid produced. I have recently shown (Gilmour, 1940) that in the termite, *Zootermopsis nevadensis,* about half the oxygen debt is repaid, and that the carbon dioxide produced during anaerobiosis is not all due to the buffering of acids.

The general habits and mode of nutrition of the primitive wood-feeding cockroach, *Cryptocercus punctulatus* Scudder, are similar in many ways to those of the lower termites. In common with them, the roach possesses a well-developed intestinal fauna of flagellates, which are responsible for the digestion of cellulose (Cleveland, 1934). It seemed likely, therefore, that a consideration of the anaerobic metabolism of this insect, which in some ways seems to occupy a position intermediate between the higher roaches and the more primitive termites, might yield information on the nature of the differences in the anaerobic processes of the two groups.

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Material and Methods

The insects were collected near Blacksburg, Virginia. Two different batches of material were used in the experiments. The first was collected in the fall of 1938 and kept in the laboratory during winter

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at a room temperature of about 22° C.; the second was collected in the spring of 1939 and kept during the summer in a constant-temperature room at 15° C. The diet consisted of moist paper towelling or filter paper.

Respiration was measured by means of Warburg manometers. Two different types of respirometer vessel were used. The first was that described by Pomerat and Zarrow (1937); the other was of the common conical design without centre-well and with a double side-arm. Respiratory quotients were measured by following oxygen uptake for a certain length of time (usually one hour) and then determining the carbon dioxide absorbed, by acidification of the alkali. Fully-grown roaches were studied throughout, one insect being used in each experiment.

Activity was reduced to a minimum by lowering the temperature and placing each insect in a small cage. The cages were made of perforated celluloid, the perforations being sufficiently large and numerous that there was no interference with the diffusion, in and out, of gases. The temperature of the experiments in which the first batch of animals was investigated was 7.5° C. For the second batch, it was necessary to reduce the temperature to 5° C. in order to eliminate all movement. This difference may have been due to the fact that the temperature at which the insects were cultured was lower in the second group than it had been in the first.

Anaerobic conditions were produced by running pure nitrogen (moist and equilibrated to the temperature of the water bath) through the respirometer vessel for a period of 15 to 20 minutes. At the end of the anaerobic period the nitrogen was replaced by CO_2-free air.

The insects were defaunated by exposing them to oxygen at 4 atmospheres pressure for 2 hours.

Experimental Results

Normal Insects

Aerobic Respiration.—The oxygen consumption of normal individuals was 46.1 cu. mm. per gram wet weight per hour at 7.5° C. (average of 70 experiments varying between 23.8 and 68.5), and 28.5 cu. mm. per gram per hour at 5° C. (average of 12 experiments varying between 20.5 and 37.5).

The respiratory quotient varied widely (0.79–1.65), the average of 30 experiments being 1.09. There were no significant differences between the results of experiments run at 5° C. and those at 7.5° C.

The production of combustible gases by the protozoa was investigated in a number of experiments, in which the insects were placed in a closed chamber and the gases present after an interval of some hours analysed with a Haldane apparatus. The protozoa of the roach were found to produce much smaller quantities of combustible gases than did those of the termite. The rate of evolution at 22° C. was about 2 cu. mm. per gram insect per hour—less than 1 per cent of the insect's oxygen uptake, whereas in the termite it has been shown to be about 10 per cent. The analyses indicated that hydrogen was not the only gas evolved, since in almost every case some carbon dioxide was formed as a result of the combustion. It was not possible, however, to arrive at any quantitative estimation of the amounts of different fractions present, since, even when the experiments were continued for such a long time that a large proportion of the oxygen present had been used up, the concentration of combustible gases in the sample analysed was usually less than 0.1 per cent.

This slow rate of evolution of combustible gases was confirmed subsequently in the anaerobic experiments, there being no significant increase in pressure when the respirometers were filled with nitrogen and carbon dioxide was being absorbed by alkali.

The high respiratory quotient is most probably due here, as in the termite, to the presence of large numbers of anaerobic protozoa.

Effects of Anaerobiosis.—When returned to air after 4 hours in nitrogen at room temperature, *Cryptocercus* remained motionless for 1 to 1½ hours, and thereafter showed increasing activity, returning apparently to normal after about three hours. An anaerobic period of 5 hours proved fatal to some, while others recovered, but none survived 6 hours in nitrogen. There was a partial recovery after 6 hours, and even to a smaller extent after 7 hours, but the insects were all dead on the day following the treatment.

Oxygen Debt.—In all the oxygen debt experiments the oxygen consumption was first followed for 2 to 3 hours, after which the air in the respirometers was replaced by nitrogen. After an anaerobic period of 1 hour, the recovery respiration was followed until a steady rate of oxygen consumption had been re-established and maintained for 2 or more hours. The results of these experiments are presented in Table I. The final rate of oxygen uptake is not far removed from the initial rate, but there is, in general, a tendency for the rate after recovery to be higher. The final rate, however, seems to be quite as constant as the initial, and has been followed for as long as 4 hours.

Cryptocercus constantly consumes during recovery an excess amount of oxygen greater than the amount of oxygen missed in anaerobiosis, and, in fact, repays the debt, on the average, three times over. By "oxygen missed in anaerobiosis" is meant the amount of oxygen which the insect would have consumed during the same period if it had been in air. The time required to effect complete recovery after only 1 hour of anaerobiosis was 4 hours.

Carbon Dioxide Excretion During and After Anaerobiosis.—Experiments on carbon dioxide excretion during anaerobiosis were performed in the same way as for a determination of respiratory quotient,

Table I

Repayment of oxygen debt in untreated insects.

The duration of anaerobiosis was approximately one hour in each case. Any slight deviations from this time have been taken into account in calculating the oxygen missed.

Experiment	Temperature °C.	Initial O_2 uptake mm³./gm./hr.	O_2 missed mm³./gm.	Final O_2 uptake mm³./gm./hr.	Excess O_2 consumed mm³./gm.	$\frac{\text{Excess } O_2 \text{ consumed}}{O_2 \text{ missed}} \times 100$
A 16 V	7.5	50	58	55	187	320
A 16 IV	"	43	50	55	133	270
A 25 I	"	41	48	47	142	300
A 25 IV	"	41	48	54	133	280
A 27 (2)	"	56	70	60	223	320
A 27 I	"	41	48	47	132	280
A 27 IV	"	39	46	52	95	210
A 27 V	"	41	48	56	191	400
A 27 (1)	"	40	50	43	167	330
B 1 (1)	"	57	57	69	106	190
B 1 (2)	"	52	52	38	91	180
Average		46	52	52	145	280
B 2 (i)	5.0	30	30	31	99	330
B 2 (ii)	"	27	27	29	79	290
B 2 (1)	"	23	23	28	80	350
B 2 (2)	"	26	26	29	112	430
B 32 (2)	"	34	34	38	123	360
Average		28	28	31	99	350

except that the air in the respirometer was replaced by a rapid stream of nitrogen before the run was started. The carbon dioxide taken up by the alkali was liberated after an anaerobic period of 1 hour in each case. The results of 4 experiments, all done at 5° C. were as follows:

(cu. mm. per gram) 79 89 78 75 average 80.

The amount of carbon dioxide excreted during anaerobiosis is thus almost three times as great as the amount of carbon dioxide produced during a similar period in air.

TABLE II

Respiratory quotients at successive one-hour periods after anaerobiosis. Untreated insects.

These figures do not represent successive determinations on single insects. Each quotient is obtained from a separate individual.

Hourly periods	1	2	3	4	5	6
	0.31	0.58	0.86	0.90	1.00	1.02
	0.34	0.80	0.84	1.00	0.82	0.92
	0.71	0.78	0.80	0.55	1.18	1.17
	0.51	0.52	0.45	0.55	1.30	0.98
R.Q.	0.46	1.12	1.04	1.05	0.99	1.14
	0.58	0.54	0.77	1.13	1.14	1.00
	0.57	0.75	0.86	0.92	0.97	1.13
	0.55	0.67	0.65	0.96	1.45	1.65
	0.59	0.92	0.97	0.76	1.18	—
	—	0.90	0.71	0.63	—	—
Average	0.51	0.76	0.80	0.85	1.11	1.13

The carbon dioxide retained during recovery was determined by subjecting the insects to nitrogen treatment for 1 hour, and then observing respiratory quotients over 1-hour periods for 6 successive hours. Since the individual variation in respiratory quotient is great, it was necessary to make many different determinations for each 1-hour period

TABLE III

Carbon dioxide retention during recovery. Untreated insects.

Hourly periods	O_2 consumed mm³./gm.	CO_2 produced mm³./gm.	Expected CO_2 production mm³./gm.	CO_2 retained mm³./gm.
1	65	33	69	36
2	72	55	76	21
3	47	38	51	13
4	45	38	49	11
5	31	35	35	—
6	31	35	35	—
			Total	81

in order to obtain significant mean values. The results of these experiments are given in Table II. There is a reduction in respiratory quotient during the recovery period. By the end of 4 hours the quotient has returned to its normal value.

In Table III is shown the carbon dioxide excretion for each hour, calculated from the respiratory quotients and the curve for average oxygen consumption. The carbon dioxide retained is determined by subtracting the actual carbon dioxide production from the theoretical, the latter being calculated on the assumption that the recovery process consists of an excess respiration having a respiratory quotient of 1.0 superimposed upon the " basal " respiration. The total amount of carbon dioxide retained is 81 cu. mm. per gram, which agrees very closely with the amount of carbon dioxide excreted during anaerobiosis.

Defaunated Insects

Aerobic Respiration.—All experiments on defaunated roaches were carried out at 5° C.

The average oxygen consumption was 44.6 cu. mm. per gram per hour (28 experiments, with results varying between 31.0 and 65.8). There is thus a decided increase over the rate of respiration of untreated insects. A small fraction of this increase is presumably due to a decrease in weight by the removal of anaerobic protozoan tissue. The average weight of defaunated insects was 0.67 gram, that of untreated insects, 0.73 gram.

The average respiratory quotient following defaunation was 0.69 (16 experiments, varying between 0.35 and 0.99). The results, obtained at intervals between the first and fifteenth days following defaunation, showed no definite trend with time.

Oxygen Debt.—The course of oxygen consumption during recovery from anaerobiosis in defaunated roaches is by no means the same as in normal insects. In Table IV are collected the results of experiments on the oxygen debt of defaunated insects.

In the roaches investigated 1 day after defaunation the excess oxygen uptake of recovery is only a little more than the oxygen missed. At 6 days after defaunation the insects repay twice the oxygen debt, while at 8 days the percentage repayment approaches even closer to the value for normal insects. The rate of aerobic respiration remains high in the insects studied at 6 and 8 days after defaunation, as it had been in those at 1 day.

In Fig. 1 the results of all the oxygen debt experiments done at 5° C. are presented graphically, as total oxygen consumed against time. All the results have been equated to the same base-line, and the oxygen consumed per unit time in the recovery period is expressed as a percentage of the initial rate. The graph illustrates the depression of the curve in freshly-defaunated insects and the return towards the normal curve in experiments done at longer intervals after defaunation.

In the course of these experiments a few insects were encountered in which defaunation was not complete. Apparently at the time of oxygen treatment they had contained cysts,[2] which had not been destroyed. When they were examined at the end of the oxygen debt experiments, the intestine was found to contain numerous cysts, although the number present was much smaller than the normal population of

TABLE IV

Repayment of oxygen debt in defaunated insects.

The duration of anaerobiosis was approximately one hour in each case. Any slight deviations from this time have been taken into account in calculating the oxygen missed.

Experiment	Temp. °C.	Initial O_2 uptake mm³./gm./hr.	O_2 missed mm³./gm.	Final O_2 uptake mm³./gm./hr.	Excess O_2 consumed mm³./gm.	$\frac{\text{Excess } O_2 \text{ consumed}}{O_2 \text{ missed}} \times 100$	Remarks
B 18 (i)	5.0	34	37	31	50	140	
B 18 (ii)	"	51	55	58	63	110	
B 18 (iii)	"	32	35	32	45	130	1 day after defaunation
B 18 (2)	"	53	57	48	82	140	
B 29 (ii)	"	59	59	66	73	120	
B 29 (iii)	"	36	36	42	57	160	
B 32 (i)	"	40	40	45	54	140	
Average		44	46	46	61	130	
B 29 (i)	5.0	38	38	40	80	210	6 days after defaunation
B 29 (2)	"	39	39	43	77	200	
Average		39	39	42	79	200	
B 32 (ii)	5.0	37	37	38	111	300	8 days after defaunation
B 32 (iii)	"	44	44	40	101	230	
Average		41	41	39	106	260	
B 18 (1)	5.0	44	48	43	166	350	Unsuccessful defaunation; cysts present
B 29 (1)	"	52	52	66	180	350	
B 32 (1)	"	46	46	53	91	200	
Average		47	49	54	146	300	
B 1 (iii)	7.5	54	54	58	41	80	Untreated; just moulted

active protozoa. These roaches behaved, as regards the repayment of oxygen debt, just as did the normal insects, repaying three times the amount of oxygen missed (Table IV).

[2] True cysts are formed in *Trichonympha*, but the corresponding stage in *Barbulanympha* is a rounded resistant form without a cyst wall. Cf. Cleveland, *et al.* (*loc. cit.*), pp. 236 and 262.

One other insect deserves special mention (Table IV, *B* 1 (iii)). It was an untreated roach, but had undergone a moult just prior to the time of the experiment. This individual showed the lowest percentage repayment of debt of all (80 per cent). Although the contents of the gut were not examined, this insect also presumably contained resistant forms, this being the means by which the protozoa are enabled to maintain themselves from one instar to the next.

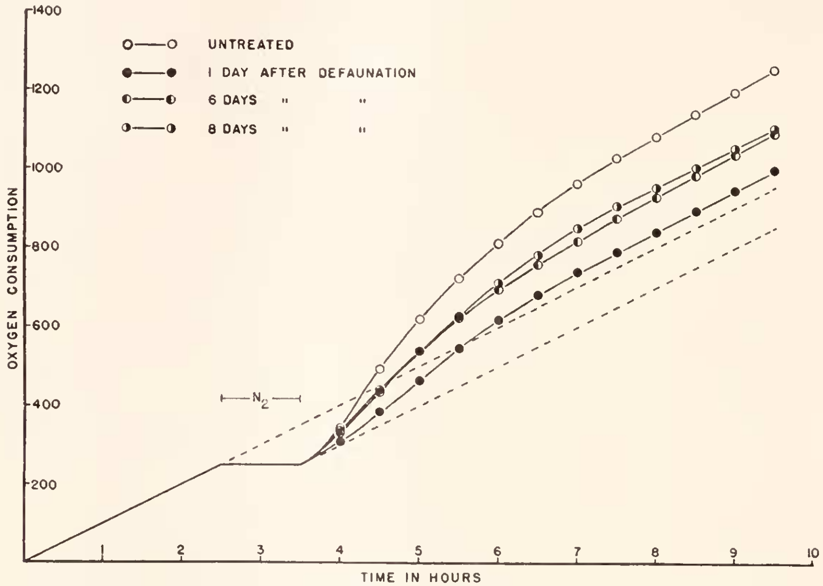

FIG. 1. Repayment of oxygen debt in normal and defaunated *Cryptocercus;* averages of all experiments at 5° C. Ordinates represent total oxygen consumed. The initial oxygen uptake per gram per hour has been made equal to 100 in each group.

Carbon Dioxide Excretion During and After Anaerobiosis.—Experiments on the excretion of carbon dioxide by defaunated insects during 1 hour of anaerobiosis yielded the following results:

(cu. mm. per gram) 84 88 72 52 average 74.

These experiments were done on insects which had been defaunated 7 days previously. The average is not significantly different from the figure for carbon dioxide excretion during anaerobiosis in normal insects. Actually one would expect it to be less than the value for normal insects by an amount equal to the carbon dioxide production of the protozoa. But since the latter would amount to only 5 to 10 cu. mm.

per gram, it would require a large number of experiments to establish such a difference.

A series of determinations of respiratory quotients at successive hours after anaerobiosis was also done on defaunated insects, and the results are presented in Table V. There are fewer experiments than in the series on normal insects and the averages consequently show a less uniform trend. These experiments were carried out either 4, 5 or 7 days subsequent to the defaunation of the insects. The average oxygen uptake of insects investigated 6 days after defaunation was therefore used in determining carbon dioxide excretion, since these figures would approximate most closely to the oxygen uptake of the insects used in the respiratory quotient experiments. The amount of carbon dioxide retained, determined on this basis, is 81 cu. mm. per gram, exactly the same as the carbon dioxide retention of normal insects.

TABLE V

Respiratory quotients at successive one-hour periods after anaerobiosis. Defaunated insects.

Hourly periods	1	2	3	4	5	6
R.Q.	0.23	0.73	0.72	0.59	0.66	0.83
	0.31	0.44	0.26	0.49	0.57	0.99
	0.22	0.64	0.70	0.54	0.66	0.50
	0.19	0.37	0.69	0.39	0.74	0.77
	0.37	0.50	0.81	0.69	—	—
Average	0.26	0.54	0.64	0.54	0.66	0.77

DISCUSSION

It is apparent that the anaerobic gaseous metabolism of *Cryptocercus* differs in almost every particular from that of *Zootermopsis*. In the first place, the carbon dioxide retained in recovery is equal, both in untreated and defaunated insects, to the carbon dioxide excreted during anaerobiosis, which suggests that the latter is derived entirely from the acidification of bicarbonate. It is thus possible that the anaerobic processes in *Cryptocercus* consist entirely of lactic acid glycolysis.

Another point of difference between the two insects is in the percentage repayment of the oxygen debt. Furthermore, there is a decided disagreement in *Cryptocercus* in the amount of repayment between normal and defaunated individuals. A suggestion of minor differences of this nature were obtained from the experiments on *Zootermopsis,* but nothing of the magnitude and regularity of the differences found in

Cryptocercus. It may be stated, broadly, that the removal of *active* protozoa from the gut in some way affects the metabolism so that the percentage repayment of oxygen debt is at first much reduced, although this effect wears off gradually. It is difficult to find an explanation for this phenomenon which would fit all the facts. It seems improbable that defaunation has produced any profound change in the nature of the anaerobic processes, since carbon dioxide excretion during and after anaerobiosis is not altered from the normal.

It is possible that the difference in the repayment of oxygen debt is associated with the increased aerobic respiration of defaunated animals. The oxygen uptake of normal insects is 29 cu. mm. per gram per hour and the carbon dioxide production is 32 cu. mm. per gram per hour, while in defaunated individuals carbon dioxide production is 31 cu. mm. per gram per hour and oxygen uptake is 45 cu. mm. per gram per hour. This suggests that the change following defaunation might have been simply the addition of some process which consumes oxygen without producing carbon dioxide. If this were the case, it would be incorrect to compare the excess oxygen uptake of recovery to an oxygen debt based on the previous aerobic respiration. That there is no change in carbon dioxide production per gram coincident with the removal of the protozoa is not surprising, since there is at the same time a loss of weight representing probably the weight of the protozoa, there being no compensation as there was in the termite, by the addition of an equal weight of non-living material.

The results from insects investigated at 6 and 8 days after defaunation and from those in which cysts were present cannot be explained on these lines, however, since the repayment of the debt was high, in spite of a high rate of oxygen uptake. Perhaps defaunation produced some change in the basal energy requirements in anaerobiosis.

The exaggerated repayment of the oxygen debt in *Cryptocercus* is interesting in the light of the conclusion of Davis and Slater (1928) that in the common cockroach all the lactic acid produced in anaerobiosis is oxidised during recovery, none being resynthesized to glycogen. Davis and Slater found only a fifth of the amount of lactic acid they had expected, and if the rate of aerobic respiration they observed was the actual basal rate, lactic acid glycolysis could have represented only part of the anaerobic processes. Alternatively, if glycolysis were the only mechanism, the aerobic respiration they observed must have been about five times as great as the actual basal value, since an anaerobic mechanism depending entirely on glycolysis, in which all the lactic acid was oxidised during recovery, would entail a repayment of the debt many times over.

The only other attempt to arrive at a quantitative estimate of the repayment of the debt in insects seems to be that of Bodine (1928) on grasshoppers. The duration of anaerobiosis was not very accurately controlled, and the repayment was sometimes less, sometimes more than 100 per cent. Bodine stated that " in carefully-controlled experiments," it could be shown to be approximately equal to the oxygen debt.

If the anaerobic metabolism in *Cryptocercus* is maintained by glycolysis, as the results suggest, the course of the oxygen uptake during recovery means that a large proportion of the lactic acid is being burned. The anaerobic metabolism of *Cryptocercus* thus resembles more nearly that of other roaches than it does that of the termite, which agrees so closely with it in the method of nutrition.

The association of a high carbon dioxide retention with a high percentage repayment of the oxygen debt in *Cryptocercus* and a low carbon dioxide retention (particularly in relation to the anaerobic carbon dioxide excretion) with a low percentage repayment of oxygen debt in *Zootermopsis* suggests that the repayment of an oxygen debt is perhaps, in fact, related specifically to the removal of lactic acid during recovery.

Summary

Fully-grown specimens of *Cryptocercus punctulatus* Scudder had an average oxygen consumption of 46.1 cu. mm. per gram per hour at 7.5° C. and 28.5 cu. mm. per gram per hour at 5° C. The average respiratory quotient was 1.09.

The production of combustible gases by the intestinal protozoa was found to be much less than that previously reported for the termite, *Zootermopsis*.

Complete exclusion of oxygen was fatal to the insects if continued for more than 5 or 6 hours.

The extra oxygen consumed during oxidative recovery from a period of anaerobiosis lasting 1 hour was, on the average, three times as great as the amount of oxygen missed. Recovery was complete at the end of 4 hours after the re-admission of air.

The carbon dioxide excreted during anaerobiosis was 80 cu. mm. per gram in 1 hour at 5° C. and was equal to the amount of carbon dioxide retained during recovery, indicating that the carbon dioxide evolved during anaerobiosis was probably all derived from the acidification of bicarbonate.

In defaunated roaches the average oxygen consumption at 5° C. was 44.6 cu. mm. per gram per hour. The respiratory quotient was 0.69.

In insects investigated 1 day after defaunation the extra oxygen consumed during recovery from anaerobiosis amounted to only 130 per

cent of the oxygen debt. At 6 days after defaunation 200 per cent of the debt was paid off, and at 8 days, 260 per cent.

Insects which had undergone the defaunation treatment, but in which defaunation was not complete, owing to the presence of resistant stages of the protozoa, had a heightened rate of oxygen consumption but behaved like normal insects, paying off 300 per cent of the debt.

The anaerobic carbon dioxide excretion and carbon dioxide retention during recovery of defaunated roaches were the same as those of untreated individuals.

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